

7h. Sect.

Thesis for M. D. degree. 1926

Wassermann Reaction in diseases
other than Syphilis

by

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1. Introduction.

The phenomenon of complement fixation was discovered by Bordet and Gengou¹ in 1901. They showed that when an antigen, a substance capable of stimulating the formation of antibodies, is mixed with its inactivated serum, the complement is firmly fixed by the combined immune body and antigen in such a way that it can no longer be found free in the mixture. If to such a mixture, an inactivated haemolytic system is added, no haemolysis takes place, since there is no free complement present to complete the haemolytic system. If, on the other hand, the original mixture contains no antibody for the antigen used, the complement is not fixed and is available for the completion of the haemolytic system thereby producing haemolysis. They² further showed that neither antigen alone nor amboceptor alone can fix complement, but this fixation is carried out by the combination of antigen plus amboceptor.

The first direct application of the test to the study of disease was made by Widal and Lesourd³ who applied it to the diagnosis of typhoid fever. Wassermann, Neisser and Bruck⁴ next applied the test to syphilis with marked success. Very shortly afterwards Detre⁵ published a similar report and with the publication of these two reports the complement-fixation test for syphilis was born.

Wassermann and his collaborators used a specific antigen i.e. a salt solution of organs of a syphilitic foetus known to contain large numbers of spirochaetes. The specificity of the test was explained on the basis of Ehrlich's side-chain theory that the absorption of complement occurred as a result of the union of an antigen

and its specific antibody.

Weygandt⁶, Fleischmann⁷, and Michaélis⁸ found that watery extracts of normal organs served to fix complement with syphilitic antibody.

Finally Landsteiner, Müller and Pötyl⁹ showed that an alcoholic extract of guinea-pig heart yielded equally good results as an aqueous extract of syphilitic foetal liver.

Alcoholic extract of normal organs is now generally used as antigen in doing the Wassermann test and it is no longer possible to say that the reaction is a direct application of Bordet's phenomenon. It is not a true antigen-antibody reaction. Notwithstanding the fact that the Wassermann test is universally recognised as a very important means of diagnosing syphilis serologically with the use of a non-specific antigen, still we are in the dark as to the mechanism of the reaction. The most that can be said is that the reaction is due to an interaction between lipotropic bodies present in the blood serum of syphilitics and lipoids in the antigen extracts.

It is of the utmost importance to know in what diseases, other than syphilis, the Wassermann test gives a positive reaction or in other words whether the peculiar lipotropic antibody is found in diseases other than syphilis. I have done the tests in Malaria, Kala-azar, Leprosy, Relapsing fever and Tuberculosis and the results obtained form the subject matter of this thesis.

The technique employed throughout the investigation was essentially the same as "Method Number Four" described in the Medical Research Committee-report¹⁰. The main differences from that method were (1) that an additional

tube containing 8 minimal haemolytic doses of complement was included, and (2) an additional control in the shape of a weakly syphilitic serum which deviates 3 minimal haemolytic doses of complement was used as well as a strongly syphilitic serum deviating 8 minimal haemolytic doses of complement.

2. Wassermann reaction in an unselected Adult male population.

I¹¹ have shown that 22 per cent of an apparently healthy Indian male adult population, taken without selection, showed latent or clinically inactive syphilis as demonstrated by the Wassermann reaction.

The importance of this investigation lies in the fact that comparative research may be instituted into the possibility of the occurrence of the Wassermann reaction for syphilis in other affections or under special circumstances and that such research is almost valueless without a basis for comparison as presented here.

The sera of 400 totally unselected Indian male patients, between the ages 20 and 60 years, who attended the pasteur Institute for antirabic treatment were tested. These patients came from various parts of India. They were of different castes and rich and poor were included at random in the examination. A positive wassermann reaction was obtained in 88 out of the 400 cases tested, which is equivalent to 22 per cent. Nine cases whose sera partially denoted 3 minimal haemolytic doses of complement, were included in the negative results owing to the fact that some normal individuals with undoubted negative syphilitic history give a \pm reaction.

The sera of 30 healthy individuals selected in the first instance because of an emphatically negative syphilitic history and because of no probability of such an infection, were examined and each and all gave a completely negative reaction.

An objection might be raised that the antirabic treatment in itself might invalidate the results obtained, and therefore the sera of 28 cases before starting treatment, of 23 cases early in treatment, and all these cases after the termination of treatment, were examined. In no case, any alteration of the wassermann reaction as a result of antirabic treatment was demonstrable.

In studying the literature regarding the value of a positive wassermann reaction in certain diseases other than syphilis one is struck with the fact that no importance has been paid to the possibility of a certain proportion of these positive findings being due to latent syphilis in the patient and not to the disease under investigation.

The following conclusions were arrived at after the examination of the sera of 400 male Indian adults :-

(1) Twenty-two per cent of an apparently healthy Indian male adult population, taken without selection, showed latent or clinically inactive syphilis as demonstrated by the wassermann test

(2) There is no reason to suppose that this population differed appreciably from a random population of the same class and age constitution. The class constitution was a very varied one.

(3) The per-centage of positive wassermann reaction

amongst the various castes and religions, does not indicate that any one caste or religion is more syphilitic than another.

3. The Wassermann reaction in Malaria.

In India where malaria is omnipresent, it is of very great importance to know whether the disease gives a positive wassermann reaction or not. In going through the literature on the subject, one is struck with the fact that no account is taken of the syphilis rate of the community and without such a basis for comparison, the reports that malaria gives a positive reaction are quite valueless. Wassermann surveys have shown that the syphilis rate is by no means small amongst people who have neither signs or history of syphilis. A very striking and instructive case is reported by McConnel¹² where a positive Wassermann reaction was obtained from a patient suffering from malignant malaria. There was a definite history of venereal sore 25 years previously. The man died of malignant malaria, and at the post-mortem examination clear evidence of syphilitic aortitis was found. If this man had denied syphilis and no post-mortem held, the case would have been regarded as a case of positive wassermann reaction due to Malaria.

A brief survey of the literature on the subject is referred to below, much of it consists of isolated observations without any basis for comparison as to the syphilis rate existent amongst the population.

Cziknawerow¹³, Manu Muscel and Vasiliu¹⁴, Fletcher¹⁵, Mathis and Heyman¹⁶, Craig¹⁷, Kimura¹⁸, Thompson and

Mills¹⁹, Levey²⁰, Johnson²¹, Kolmer²², Chambers²³, and Lloyd and Mitra²⁴ all state that Malaria does not give a positive Wassermann reaction.

Boehm²⁵ states that 16 out of the 46 cases of Malaria gave a positive wassermann reaction and recommends that in a malaria patient who is also suspected to be syphilitic the wassermann reaction should not be tried till the malaria is cured. Meir and Bongfiglio²⁶ are reported by Wassermann to have found that so long as malaria parasites are present in the blood the wassermann reaction is positive. Thompson²⁷ has found a positive wassermann reaction during the stage of fever and parasites, the reaction becoming negative on the disappearance of fever and parasites. Ferrari and Gioseffi²⁸ found 16.6 per cent positive in 46 cases of malaria which were clinically non-syphilitic, and they confirmed Boehm's observation that the positive reaction becomes negative under quinine treatment. de Hann²⁹ also found that some of the positive reactions became negative under quinine treatment. Meyerstein³⁰ states that the wassermann reaction was often positive in Tertian Malaria during the first few days after an attack of fever and that the reaction becomes negative simultaneously with the disappearance of the parasites under quinine treatment. Hehewerth and Kop³¹ examined series of Javanese soldiers in Batavia and they have come to the following conclusions:-

(1) Malaria in Javanese causes a positive wassermann reaction in over 50 per cent (2) this reaction disappears as a rule under quinine treatment. These positive reactions seem to have occurred when they used non-inactivated sera. It is shown in the League of Nations Health organi-

sation report on the serodiagnosis of syphilis³² that non-inactivated sera give false positives.

I³³ have examined the sera of 84 cases of undoubted malaria, all Indian males between the ages of 20 and 45 years under the following conditions :-

(1) during the cold stage (2) during the hot stage (3) in chronic cases (4) in cases about to be discharged from the hospital as cured (5) before and after quinine treatment.

1. Examination of blood during the cold stage.

Six cases in all were examined and none of them gave a positive wassermann reaction. The blood smears of these cases taken during the hot stage showed benign tertian parasites.

2. Examination of blood during the hot stage.

Fifty-two cases in all were examined. Blood smears were examined in all cases for the presence of malaria parasites. 28 of them showed benign tertian parasites, 19 malignant tertian parasites, and 5 did not show any parasites. Of the 28 cases showing benign tertian parasites, 4 gave a very strong positive reaction, of the 19 cases showing malignant tertian parasites, 1 gave a fairly strong positive Wassermann reaction and of the 5 cases that did not show any parasite, 1 gave a strong positive wassermann reaction. All the six cases which gave a positive wassermann reaction gave a definite history of recent syphilis and also showed scars on glans penis.

3. Examination of blood in chronic cases.

12 cases were examined. All of them had large spleens and pronounced anaemia. 6 of these cases showed, malignant

tertian parasites and the other 6 did not. Out of these 12 cases, only 1 gave a strongly positive reaction and the other 11 were all negative. The blood smear of the patient who gave a positive reaction did not show any malarial parasite. Syphilitic history was denied by the patient, but he had a definite scar on the glans penis.

4. Examination of blood of patients who were about to be discharged from the hospital as cured.

14 recent cases of Malaria who were under quinine treatment were examined. 12 of them had shown benign tertian parasites and the other 2 malignant tertian parasites. All the 14 gave a negative wassermann reaction.

5. Examination of blood before and after quinine treatment.

All the 52 cases examined during the hot stage had no quinine treatment. Of these 52 cases, 6 gave a positive wassermann reaction. These 6 cases were put on quinine treatment for two weeks - 210 grains during the first week and 140 grains during the second week - and then their sera were tested and the results obtained were just the same as before treatment.

Besides these 84 cases of Male Indian adults, I examined the sera of 14 Indian children, between the ages of 8 and 13 years, during the hot stage and found they all gave a negative wassermann reaction.

These results prove that the wassermann test is not positive in Malaria and that the large proportion of positive results in the early literature of the test must have been due to improper technique. Altogether 98 cases were examined out of which 7 gave a positive wassermann

reaction i.e. 8.3 per cent.

I have shown that 88 cases out of 400 of an unselected, apparently healthy Indian male adult population gave a positive Wassermann reaction i.e. 22 per cent. The positive reaction of 8.3 per cent, obtained in an Indian male adult population affected with malaria, is far below the syphilis rate of 22 per cent. Moreover all these malaria cases giving a positive Wassermann reaction showed definite signs of previous syphilitic infection.

I, therefore, conclude that malaria does not cause a positive Wassermann reaction, and that quinine administration does not affect the Wassermann reaction.

4. Wassermann Reaction in Kala-azar.

Kala-azar is widespread in India in the eastern portion of the Indo-Gangetic plain and the Brahmaputra valley, i.e., Behar, Bengal & Assam including sylhet.

Sutherland and Mitra³⁴ examined the blood of 38 cases of Kala-azar. Of these 38 cases 10 gave a positive and 28 a negative reaction. They further state that out of the 10 positive cases, only 2 gave a more than slightly positive reaction. The other 8 cases must have given a + reaction and these cannot be included under those who gave a definitely positive reaction. So the positives are only 2 out of the 38 cases, giving a per-centage of 5.3.

I³⁵ have examined the sera of 30 kala-azar patients with the object of finding out whether this disease gives a positive Wassermann reaction. The smears made from the splenic puncture at the time blood was collected for was-

sermann test showed Leishman-Donovan bodies. Of these 30 cases, 6 gave a positive wassermann reaction, thus giving a percentage of 20 positives. 4 out of these 6 cases gave a recent history of syphilis. This percentage falls within the limit of the syphilis rate of 22 percent.

Conclusion.

Kala-azar infection is not a cause of positive wassermann reaction.

5. Wassermann Reaction in Leprosy.

Eitner³⁶ was the first to report in 1908 a positive wassermann reaction in leprosy with an alcoholic extract of guinea-pig heart as antigen and in the same year Weihselman and Weier³⁷ and by Slatineau and Danielopolu³⁸ published similar reports of reaction by using the original wassermann antigen.

Babes³⁹ reported in 1909 that nodular leprosy gave a positive and anaesthetic leprosy a partial or negative reaction and similar reports were made by Eliasberg⁴⁰, Bruick and Geisner⁴¹, and Ehler and Bourret⁴².

Fletcher⁴³ in 1919 examined 100 lepers finding 22 positive reactions. Haseltine⁴⁴ has given the results obtained by various workers (Table shown at the end of the paper). He himself obtained 25 per cent of positive reactions by using Neil's⁴⁵ method and 21.75 per cent by using Kolmer's⁴⁶ method. He concludes that there is a tendency in leprosy to the formation of reagin capable of causing non-specific complement fixation.

Kolmer and Denny⁴⁷ report that in a series of 159 cases of leprosy, clinical or serologic evidence of syphi-

lis was found in 27. In non-syphilitic lepers, the Wassermann reaction by a new technique was uniformly negative.

Sutherland and Mitra⁴⁸ state that they themselves found that 4 out of 14 individuals suffering from anaesthetic form of leprosy, and 4 out of 20 affected with other forms, gave a positive Wassermann reaction.

Lloyd, Muir and Mitra⁴⁹ examined 228 Indian adults who were all undoubted cases of leprosy. They obtained 63 per cent positives in nodular cases, 47.4 per cent in mixed cases and 27 per cent in anaesthetic cases. All the types together gave 41.7 per cent positive.

With a view of eliminating syphilis as far as possible, they examined the Wassermann reaction in a series of 58 leper children and found 100 per cent positive in nodular cases, 80 per cent in mixed cases and 47 per cent in anaesthetic cases. All the types taken together gave 62 per cent positives. The number of cases examined in the nodular type were only 4 and all these gave positive reaction. All these 58 leper children were carefully examined for syphilis and only 4 showed suspicious lesions and out of these 4, only 1 gave a positive reaction.

As a control series they examined 46 untainted children whose parents or guardians were lepers and who came from the same community as the leper children above referred to. These untainted children gave 17 per cent positive which is markedly different from the 62 per cent in the leper children.

⁵⁰
Cooke states "It is obvious however that while a certain proportion of positive fixations may be accounted

for by a coincident syphilitic infection, the percentage is entirely too high to be explained by this factor alone, and we must conclude that infection with Hansen's bacillus may cause the appearance in the blood of substances capable of giving a positive wassermann reaction, and that these substances are more frequently present in cases with skin lesions than in cases with nerve lesions alone."

The sera of 100 cases of undoubted leprosy have been examined by me⁵¹. They were all Indian males between the ages of 20 and 60 years. Of these 100 cases, 34 were of the nodular, 52 of the anaesthetic and 14 of the mixed form, and the number of positive wassermann reaction for these several types were 17, 16 and 8 respectively. All the types taken together the percentage of positives was 41.

The sera of 12 healthy children born of leper parents were examined by me. The ages of these children were from 11 months to 15 years, and they all gave a negative reaction. Six of these children were from a leper father, 4 by the first wife and 2 by the second. The first wife died from leprosy. The blood of the father and the second wife, both lepers, gave a strong positive wassermann reaction. There was no history of abortion or miscarriages either in the first or second wife and father totally denied of any specific infection. Two out of the remaining 6 children were from leper parents. The mother gives a strong positive wassermann reaction. They have had 8 children, one of them died of leprosy when 17 years of age, the others are alive with no evidence of leprosy or any other disease. There was no history of abortion or miscarriage in the mother. Two out of the remaining

4 children were from undoubted leper parents. The father gave a strongly positive wassermann reaction but the mother gave a negative reaction. The bloods of the parents of the remaining 2 children was not available.

The absence of a positive wassermann reaction in these children, which to a large extent is found in those suffering from leprosy, constitutes evidence to the view that this positive reaction is characteristic of the disease and not due to underlying syphilitic infection. Moreover, the fact of these leper parents having healthy children whilst giving a strong positive wassermann reaction, is further evidence against the reaction being syphilitic.

Conclusions.

(1) 41 per cent positive obtained in lepers is much higher than the syphilis rate of 22 per cent obtained in the unselected male adult Indian population and so it is to be inferred that the reaction is a sign of leprosy and not merely of syphilis.

(2) Further evidence of the characteristic nature of the wassermann reaction in leprosy is afforded by the fact that lepers who gave strongly positive wassermann reaction had been able to reproduce healthy children, i.e. children without any signs of leprosy or of syphilis.

Table, compiled by Haseltine, giving Wassermann results obtained by various workers in leprosy cases.

Year	Author	No. of cases.	Positive.	Negative
1908	Eitner	1	1	0
1908	Wechselman, Meier	1	1	0
1908	Slatineau, Denielopolu	21	16	5
1908	Meier	22	10	12
1908	Jundell, Almquist, Sandmann	26	8	18
1909	Eliasberg	50	28	22
1909	Frugoni	4	3	1
1909	Bruck, Geissner	10	5	5
1910	Ehlers, Bourret	47	45	2
1910	Thomson, Bjarnhjedinson	50	11	39
1910	Fox	60	34	26
1910	Montesonto, Satirades	48	31	17
1911	Bloomberg	21	3	18
1912	Photinos, Michaelides	204	115	89
1913	Rocomara	19	14	5
1913	Clegg	24	11	13
1915	Nakajo, Asakura	33	26	7
1915	Fletcher	100	22	88
1915	Sutherland, Mitra	34	11	23
1917	Honey	11	9	2
1919	Iyengar	100	41	59
		<hr/> 886	<hr/> 445	<hr/> 441

6. Wassermann Reaction in Tuberculosis.

Letulle⁵² states that 19 per cent of 346 tuberculosis inmates of Boucicaut Hospital gave a positive response to wassermann test.

Snow and Cooper⁵³ state, as result of examination of 290 tuberculosis patients, that in the absence of syphilis, tuberculosis does give a positive wassermann reaction if a non-cholesterinised antigen is used. By using cholesterinised antigen they got 31 per cent positive.

Edward Von Adeling⁵⁴ found 17 out of 195 tuberculosis cases gave a positive wassermann reaction, a percentage of 8.7.

Cooper⁵⁵, as a result of the routine serologic blood examination of 1395 men and 1399 women residents of the Chicago Tuberculosis Sanatorium, found a definite positive wassermann reaction in 7.2 % of men and 5.8% of the women.

Kilduffe⁵⁶ obtained positive reaction in 10 out of 104 cases of pulmonary tuberculosis. One case had a history of chancre eight years and another 35 years before; a third had a typical secondary eruption at the time of the test which later cleared up under specific treatment. He concludes that with a technically correct and carefully applied method, positive reactions do not occur in tuberculosis in the absence of syphilis.

Kolmer⁵⁷ found no positive reaction in tuberculosis in the absence of syphilis. He used a cholesterinised antigen. Downs⁵⁸ in 153 cases found 38 positive reactions in none of which syphilis could be excluded.

Boas and With⁵⁹ obtained a positive wassermann reaction in 3 out of 1343 cases of tuberculosis skin affections.

The response was always negative in 758 cases of surgical tuberculosis and in 376 cases of pulmonary tuberculosis.

I⁶⁰ have examined the sera of 70 tuberculous patients with the object of finding out if this disease was responsible for a positive wassermann reaction. Of these 70 cases, 40 were of pulmonary tuberculosis, 14 were cases of tuberculosis of the lymphatic glands, and 16 were cases of abdominal tuberculosis. Fourteen out of these 70 cases (8 out of 40 pulmonary cases, 3 out of 14 of tuberculosis glands and 3 out of 16 of abdominal) gave a positive wassermann reaction, a percentage of 20. Three out of these 14 cases gave a definite history of recent syphilis and 8 admitted exposure to infection but had not shown any signs of syphilis.

All the 70 cases examined were Indian male adults, between the ages of 20-50 years.

Conclusion.

(1) In view of the fact a syphilis rate of 22 per cent was obtained amongst an unselected, apparently healthy, Indian male adult population, I infer that a figure of not more than 20 per cent, occurring in an unselected male adult population suffering from tuberculosis, must be due to the existence in this population of latent or clinically inactive syphilis.

(2) Tuberculosis is not responsible for a positive wassermann reaction.

7. The Wassermann Reaction in Relapsing fever.

Korshum and Leibfried⁶¹ examined the sera of 50 relapsing fever cases and found 56 per cent positive when syphilitic antigen was used and 96 per cent positive with spirochaetal antigen.

Fairley and Sullivan⁶² tested the sera of 10 relapsing fever cases during the pyrexial period and of 22 cases during apyrexial period. One gave a positive reaction during the pyrexial period and 3 during apyrexial period.

Roaf⁶³ found that 11 out of 18 of his cases of relapsing fever cases gave a positive reaction and states that a transient positive wassermann reaction may be found to be a constant phenomenon during the acute stage of this disease.

I have examined the sera of 38 cases of relapsing fever. 34 of these were Indian male adults between the ages of 20-50 years and the remaining four were children between the ages of 1-4 years. In all these cases, films were examined for the spirillum before taking the blood for the wassermann reaction and the findings were positive in all.

Thirty out of 34 adults and all the four children gave a strongly positive wassermann reaction :- a percentage of 90.

All these cases were given each an injection of Neo-salvarsan and the temperature came down to normal, 2nd to 3rd day after the injection.

18 cases out of the 34 adults who gave positive wassermann reaction during the pyrexial period were all examined on the 4th, 8th, and 12th day after the temperature

had come down to normal. 4 cases gave a negative result on the 4th day, 9 on the 8th day and 5 on the 12th day. Two out of the 4 cases giving a negative reaction on the 4th day had a relapse and the blood films showed the spirillum. The sera of these 2 cases again gave a strong positive reaction.

Conclusion.

Relapsing fever is a cause of positive wassermann reaction. The transient character of this reaction distinguishes it from the reaction due to syphilis.

8. Summary and Conclusions.

Since the introduction of the wassermann test as an aid to the diagnosis of syphilis, positive reactions have been reported in almost all non-syphilitic disease known to medicine. In a great majority of these findings, these are due to inherent and unrecognised technical errors. Furthermore no investigation was done as to the syphilis rate in the general community of which these cases of non-syphilitic disease formed a part. Statistics relative to the incidence of positive reactions in diseases, other than syphilis, are valueless unless we have a syphilis rate to compare with and also complete data relative to the method employed in carrying out the tests. If the percentage of positive reactions obtained in any disease by the accepted methods of the day is much above the syphilis rate, it should be taken that the disease itself is the cause of the positive reaction and not syphilis.

An apparently normal healthy male adult Indian population gave a syphilis rate of 22 per cent as judged by the wassermann reaction.

Malaria, Tuberculosis and Kala-azar gave 8.3, 20 and 20 per cent of positive respectively and these figures are lower than the syphilis rate. I infer that these diseases are not responsible for a positive wassermann reaction.

Positive reactions of 90 per cent in relapsing fever and 41 per cent in leprosy are much higher than the syphilis rate of 22 per cent and I, therefore, infer that both these diseases give a positive wassermann reaction. The positive reaction in relapsing fever is of a transient nature and if this reaction persists it should be concluded that it is due to syphilis.

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